# Rate of Stratum Corneum Formation in the Perinatal Rat

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During late gestation, the fetal rat exhibits marked hyperplasia of the interfollicular epidermis and accelerated cornification in preparation for birth. In this study, we utilized simple morphometric techniques to provide quantitative estimates of the rate of stratum corneum (SC) formation during the perinatal period. Cryostat sections of dorsal epidermis from rat pups between -48 and +72 h of age were expanded under alkaline conditions and the number of corneocyte interfaces counted from photomicrographs. This method yielded the following regression lines: prenatal, y = 0.19x +13.07, r = 0.93; postnatal, y = 0.13x + 13.00, r = 0.93, where y = number of SC layers and x = age in hours. Lack of desquamation was assured by the postnatal persistence of the granular periderm. Adhesive stripping of the epidermis followed by phase-contrast microscopy revealed intact mono-

rom a biomaterials perspective, the mammalian stratum corneum is a cohesive, rugged yet flexible, polymeric film that is relatively impermeable to water loss. This biopolymer is both self-cleaning (through the process of desquamation) and self-renewing (through the addition of new polymer units). The latter property is the end result of a highly orchestrated series of differentiative events within the interfollicular epidermis [1,2]. These events include both the regulated synthesis of specific proteins such as keratins, filaggrin, and involucrin [3] as well as the generation of neutral lipid-enriched material packaged into distinctive structures called lamellar bodies [4]. The dynamic and quantal process by which these epidermal proteins and lipids assemble into the overlying stratum corneum is generally termed "cornification."

One approach to understanding cornification is to focus on potential factors regulating the *rate* at which lipid-rich keratinocytes in the uppermost granular layer undergo transition to young corneocytes in the lowermost stratum corneum. At the cellular level, this transition is spatially discrete and characterized by the distinctive ultrastructural appearance of the so-called "transitional" cell [5]. The paucity of transitional cells observed on routine photomicrographs is generally thought to be secondary to the rapidity of the transitional cell stage (minutes to hours) [6].

In this work, simple, proved morphometric techniques of stratum

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Abbreviations:

EGF: epidermal growth factor

layer sheets of SC. Quantitation using a computerized image analysis system gave an average corneocyte surface area in the newborn rat of  $1908 \pm 36 \,\mu^2$  (mean  $\pm$  SEM). Treatment of neonatal rats with epidermal growth factor (500 ng/g BW) increased the number of SC layers over the first 24 h of life (p < 0.05) and resulted in marked hyperkeratosis by one week of age. These results allow the following conclusions: 1) in the prenatal rat, SC forms at a rate of one layer every 5 h; 2) postnatally, SC formation slows to one layer every 8 h; 3) to support normal corneum synthesis at birth, dorsal keratinocytes must enter transition at a rate of approximately 3 cells/ second/cm<sup>2</sup> of body surface; and 4) treatment with exogenous EGF augments the rate of terminal differentiation of perinatal rat epidermis. J Invest Dermatol 100:400-406, 1993

corneum analysis have been applied to an *in vivo* model to provide estimates of the kinetics of transitional cell formation. The perinatal Sprague-Dawley rat was chosen because the epidermis of this species, like other rodents, exhibits a surge of hyperplasia of the interfollicular epidermis before birth associated with the rapid development of a well-formed, multi-layered stratum corneum. Data are presented on the influence of ontogenetic age, body site, and the ability of exogenous epidermal growth factor (EGF) to affect the rate of stratum corneum formation.

### MATERIALS AND METHODS

Animal Handling and Growth Factor Treatment Timed gestation Sprague-Dawley rats were obtained on the fifteenth day of pregnancy from Zivic Miller Laboratories, Inc. (Zelienople Park, PA). Animals were housed with a 12-h light/dark cycle and given routine laboratory chow ad libitum. Mothers routinely delivered on day 22 of gestation. Newborn pups were undisturbed until the time of experimentation. Neonatal rats were sacrificed by an intracardiac injection of Nembutal (Abbott Laboratories, North Chicago, Illinois). Fetal animals were delivered by Caesarian section on gestational day 19-21 following anesthesia of the mothers with methoxyflurane (Pitman-Moore, Washington Crossing, New Jersey). For experiments requiring treatment with EGF, pups were given subcutaneous injections of the growth factor or normal saline as described previously [7]. All animal experimentation and protocols were approved by our Institutional Animal Care and Use Committee.

**Preparation of Skin Sections for Cryosectioning** Late fetal or early neonatal Sprague-Dawley rats (Zivic Miller Laboratories) were sacrificed by intracardiac injection of sodium pentobarbitol (Nembutal). On the dorsal surface of each animal, two horizontal

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lines were drawn using a fine tip marking pen, 5 mm anterior to the base of the tail and 10 mm above that line. Dorsal skin sections were excised over a wide area using sharp scissors so that demarcated lines lay within the rectangle. Adherent fat and blood vessels were gently removed from the undersurface of the dermis with saline-soaked cotton swabs. A 5-mm-wide strip was then cut out along the midline of the animal using a sharp scalpel.

Sections were mounted using a cork disc measuring 15-20 mm in diameter and 5 mm thick as described by MacKenzie [8]. Using a scalpel, a vertical incision was made approximately 2 mm thick into the surface of the disc. A plastic coverslip was inserted into the incision against which the tissue specimen was oriented and flattened with the stratum corneum side up. Embedding media (OCT compound, Miles Inc., Elkhart, IN) was poured over the specimen and the cork disc rapidly frozen by immersion in liquid nitrogen for 5 seconds. After the specimen was frozen, the plastic coverslip was gently bent from side to side and removed. The cork disc bearing the specimen was attached to the surface of a cryotome chuck with embedding media and frozen by immersion in liquid nitrogen. The specimen was sectioned with a cryotome (Cryostat MHR, Slee Technik, Mainz, Germany) into 6-micron-thick pieces and placed on a microscope slide (3-4 sections per slide). The slides were stored at  $-20^{\circ}$ C until examination.

Alkaline Expansion of Stratum Corneum and Counting of **Corneum Layers** The microscope slides bearing the specimens were stained in filtered 0.5% methylene blue in 95% ethanol for 30 seconds followed by a gentle rinse in double distilled water. Excess stain was removed from the back and sides of a slide with absorbent tissue (Kimwipe, Kimberly Clark, Rowell, GA). A few drops of half-strength Sorensen-Walbum buffer, pH 12.5, was applied to the specimen for 20 seconds essentially as described by MacKenzie and Zimmerman [9]. A cover glass was applied and excess buffer was removed with absorbent tissue. Photomicrographs of the expanded stratum corneum were taken with a Zeiss MC63 photomicrographic camera generally using the  $40 \times$  objective and standard black and white print film (Kodak TMAX-100). Photomicrographs were examined directly on the resulting contact sheet using a Wild M3C dissecting microscope (Heerbrugg, Switzerland). On each section, perpendicular lines were drawn between the epidermal basement membrane and the overlying periderm using a straightedge. The average number of stratum corneum layers was determined by direct counting of the number of cellular interfaces within the stratum corneum. The first interface was defined as the upper membrane leaflet of the lowermost corneocyte and the last as the interface between the uppermost corneocyte and the periderm. An average of 9-15 separate areas were counted on each specimen to arrive at the final number of layers. Special attention was given to corneocyte layers just beneath the periderm (generally the thinnest and least expanded layers) and to sites of interditation of adjacent corneocytes (if the perpendicular line passed through an area of overlap, each cell interface was counted to determine the average number). Only sections in which the overlying periderm remained intact were included in the calculations.

**Determination of Average Corneocyte Surface Area** Stratum corneum specimens were collected on microscope slides using clear, adhesive tape (Scotch Transparent Tape 600, 3M Company, St. Paul, MN) mounted on the slide with the sticky side up. The slide was gently pressed over the low dorsal epidermal surface of the rat pup and removed with a slow rolling motion from left to right. This process was repeated three times with each tape-stripping product deposited on a clean, adjacent area of the microscope slide. A drop of distilled water was then added directly to the tape-stripped material for 60 seconds and removed with a capillary tube. Specimens were then stable for examination by phase-contrast photomicroscopy.  $5'' \times 7''$  glossy photomicrographs of dry, tape-stripped stratum corneum were obtained following phase-contrast photomicroscopy and the outlines of individual corneocytes traced directly onto the digitizing tablet of a ZIDAS image-analysis system (Carl

Zeiss, Inc., Thornwood, NY) programmed to determine surface area.

**Calculation of Corneocyte Kinetics** The rate of stratum corneum formation was determined according to the relation

$$T = mc = dy/dx (A/s),$$

where T is the rate of transitional cell formation, or, equivalently, the rate at which new corneocytes are added to the developing stratum corneum; m is the rate of corneum stratification; and c is the average corneocyte packing density per layer. The variables m and c were derived as follows.

i) m, the rate of corneum stratification, was determined from the regression formulae, y = mx + b, where y is the number of corneum layers and x is the age of the rat in hours. Differentiating y with respect to x,

$$dy/dx = m$$
,

where m is the rate of formation of new layers giving rise to the stratum corneum and 1/m is the time to form one layer of corneum.

ii) c, the average corneocyte packing density per layer, was determined from the equation

$$c = A/s$$
,

where A is the epidermal surface area and s is the average surface area of individual corneocytes. In this ratio, A may be the total body surface area [27] or, alternatively, a standard area, e.g., 1 cm<sup>2</sup> over a representative region such as the dorsum; s is measured from phasecontrast photomicrographs of individual corneocytes following tape stripping as described.

Scanning Electron Microscopy The effect of exogenous EGF treatment on skin surface morphology in the postnatal rat was assessed by scanning electron microscopy of the epidermal surface of whole skin sections (dermis + epidermis) dissected from the lower dorsum of 7-day-old littermate rat pups. Prior to examination on day 7, the pups received a treatment regimen consisting of subcutaneous injections of saline or EGF (50 or 500 ng/g BW) on postnatal days 0-3. Following sacrifice, a leather punch was used to obtain circular sections measuring 12.7 mm in diameter. Skin sections were mounted on solid aluminum stubs, dried overnight in an incubation oven (40°C), and sputter-coated for scanning electron microscope (Peabody, Mass). Specimens were oriented so that rostral/caudal axes were easily determinable.

**Statistical Analysis** Linear regression analyses were performed on prenatal and postnatal samples using a Lotus 123 software system. Statistical comparisons between groups were determined by Student t test. A p value less than 0.05 was considered significant.

#### RESULTS

Ontogeny of Stratum Corneum Formation Alkaline expansion of frozen sections of dorsal epidermis at different ages allowed assessment of stratum corneum development in the perinatal rat (Fig 1A - C). The number of layers of stratum corneum was counted directly from photomicrographs and the data plotted as a function of ontogenetic age (Fig 2) or body weight (Fig 3). Regression lines were calculated for the prenatal and postnatal periods to derive estimates of the number of layers of stratum corneum formed per unit time. Lack of desquamation was assured by the persistence of the granular periderm throughout the period of investigation (Fig. 1). Only sections with the periderm intact were included in the regression line calculations. The results demonstrated a significant increase in the slope of the prenatal regression line (p < 0.05), indicative of a faster rate of stratum corneum formation during the period immediately preceding birth. Plotting the data as a function of body weight yielded similar results (Fig 3, p < 0.05). Additional paired samples were also obtained comparing the dorsal and ventral epidermis and the results plotted against body weight (Fig 4). No difference in the slopes of the resulting regression lines was noted. The







**Figure 1.** Ontogeny of stratum corneum formation in the perinatal rat. Frozen sections of dorsal epidermis were stained with methylene blue and the stratum corneum visualized by expansion with alkaline buffer according to the method of Mackenzie [9]. *A*) Fetal day 20 (term equals 22 d), the stratum corneum consists of 3-4 cell layers underlying a well formed periderm. *B*) Day of birth (day 0): the stratum corneum has increased in thickness; the epidermis is well formed and the periderm forms a continuous external granular layer. *C*) Postnatal day 3: the stratum corneum has continued to increase in number of layers; the periderm is discontinuous. *Bar*, 12.4  $\mu$ .



**Figure 2.** Number of stratum corneum layers versus ontogenetic age. Data are calculated using birth as an *a priori* demarcation point such that prenatal (-48 h to 3 h of age) versus postnatal ( $\geq 3$  to 74 h of age) regression lines have been determined. The total number of pups examined is given in parentheses.

number of stratum corneum layers in the ventral samples, however, was consistently lower (by 1-2 layers) than the paired dorsal samples (p < 0.01).

**Determination of Corneocyte Surface Area** Phase-contrast microscopy was used to examine monolayer sheets of stratum corneum obtained from the dorsal epidermis of neonatal rat pups (Fig 5). Following removal of the periderm (first tape strip), the stratum corneum sheet obtained on the third tape strip was photographed and the surface area of individual corneocytes determined by computerized image analysis of the resulting photomicrographs. The results showed variation between litters (Table I) with a tendency for corneocyte surface area in the older postnatal pups to be slightly *less* than in the younger pups. This finding runs counter to the hypothesis that somatic growth, by increasing body surface area, leads to expansion of laterally fixed corneocytes.

In experiment 4, the effect of body site on corneocyte surface area was examined. The average surface area for corneocytes taken from the dorsal epidermis of 45-hour-old rat pups was  $1587 \pm 28 \mu^2$ (mean  $\pm$  SEM) compared to  $1802 \pm 33 \mu^2$  for the ventral epidermis, whereas corneocytes over the eye and ear appeared smaller (data not shown). Knowledge of the average surface area for cor-



**Figure 3.** Number of stratum corneum layers versus body weight. The data correspond to the pups examined in Fig 2. Prenatal versus postnatal regression lines were calculated based on age as described. The scatter in the data in the vicinity of 6-7 g most likely represents unequal postnatal growth rates shortly following birth.



**Figure 4.** Number of stratum corneum layers determined from paired samples of dorsal and ventral epidermis (n = 48). Combined prenatal and postnatal regression lines have been calculated for ease of comparison. Of note, the ventral specimens parallel the dorsal samples but at a slightly lower (1–2 layers) level.

neocytes covering the dorsal epidermis was utilized to develop a kinetic model of transitional cell formation rate.

Calculation of Corneocyte Kinetics According to the model proposed (see Materials and Methods), the rate of stratum corneum layer formation (m) multiplied by the average number of corneocytes per layer (c) is equivalent to the rate at which dorsal keratinocytes enter transition (T). This model assumes a one-to-one correspondence of transitional cells and new corneocytes (i.e., every transitional cell in the upper stratum granulosum progresses to form a new corneocyte in the lower stratum corneum). Applying this model to the late gestational rat, the rate of layer formation giving rise to the dorsal stratum corneum was calculated from the formula (Fig 2) y = 0.19x + 13.07, where the rate of corneum stratification is m = dy/dx = 0.19 layers/h, and the time to form one layer of corneum is 1/m = 5.3 h. The average corneocyte surface area over the dorsum of a rat at birth (6 h of age) was 1908  $\mu^2$  (Table I) = s. Taking the average surface area (A) of a 6.5-g newborn rat to be 25.6 cm<sup>2</sup> or, equivalently,  $2.56 \times 10^9 \,\mu^2$  [10], and substituting values in the relation c = A/s (see Materials and Methods), gives c = $1.3 \times 10^6$  cells. This is the average number of corneocytes per layer



**Figure 5.** Phase-contrast photomicrograph of stratum corneum from a neonatal (24-h old) rat pup. Monolayer specimens of stratum corneum were prepared for photomicroscopy by adhesive stripping of dorsal epidermis onto clear glass slides. Tissue preparation consisted of removal of the periderm (first tape strip) and examination of the stratum corneum sheet obtained on the third tape strip. *Bar*, 56  $\mu$ .

needed to cover a newborn rat (equivalent to  $5.2 \times 10^4$  cells per cm<sup>2</sup>). Substituting values in the relation T = mc (see *Materials and Methods*), gives a rate of stratum corneum formation of 0.19 layers/ $h \times 1.3 \times 10^6$  cells/layer =  $2.5 \times 10^5$  cells/h or, equivalently,  $2.4 \times 10^5$  transitional cells/cm<sup>2</sup>/d or 3 corneocytes/cm<sup>2</sup>/second. Strictly, this rate is applicable to the dorsal epidermis only. In areas, such as the ventral epidermis, where the average corneocyte size appears larger (Table I), the rate of transitional cell formation will be slightly less. On the other hand, in areas where the corneocyte size is smaller than the dorsal epidermis, the cornification rate will be slightly greater.

Effect of Exogenous EGF on Epidermal Development Table II demonstrates the effect of a single dose of exogenous EGF (500 ng/g body weight) on stratum corneum layer formation in the newborn rat. EGF was administered shortly following birth and the number of stratum corneum layers counted 24 h later. EGF treatment resulted in the formation of one additional layer over the 24-h period examined. EGF treatment also resulted in significant somatic growth retardation, with, hypothetically, a consequent decrease in estimated surface area (Table II). These results posed the following question in cell kinetics: is the number of new corneocytes "saved" by the decrease in surface area sufficient to counterbalance the increase in transitional cells required to form one additional layer of stratum corneum following EGF treatment?

According to the data shown in Table II, the diminution in surface area for the EGF-treated pups compared to controls would be approximately 2.7 cm<sup>2</sup>. Given an average corneocyte surface area of 1620  $\mu^2$  at 1 d of age (Table I), this translates to a total of  $1.7 \times 10^5$ corneocytes. Assuming three new layers of stratum corneum are laid down over a 24-h period after birth (1/m = 8 h per layer), control pups would require  $3 \times [1.7 \times 10^5]$  (or  $5.1 \times 10^5$ ) more corneocytes than the EGF-treated animals to account for their increased surface area. Based on similar calculations, the average number of corneocytes needed to form one additional layer of stratum corneum in the EGF-treated rat would be approximately  $1.7 \times 10^6$  cells. Subtracting  $5.1 \times 10^5$  from  $1.7 \times 10^6$  gives a total of  $1.2 \times 10^6$ additional cells entering transition in the EGF-treated group during the 24-h period under study. We provisionally conclude, therefore, that the somatic growth retardation elicited by EGF is insufficient to account for the observed increase in stratum corneum formation and that treatment of the newborn rat with exogenous EGF significantly augments the rate of terminal differentiation of the epidermis.

Neonatal administration of EGF also leads to "long-term" effects on the morphology of the epidermal surface. Figure 6A-C shows scanning electron micrographs of the dorsal epidermis of 7-day – old littermate rats following treatment with saline or EGF on postnatal days 0-3. EGF induced a dose-dependent hyperkeratosis of the interfollicular epidermis and a characteristic curvilinear growth pattern of monotrichs.

#### DISCUSSION

Over the past 15 years, it has become clear that the view of the stratum corneum as a "dead" or "passive" envelope is better replaced with the concept of a dynamic, adaptive surface membrane that interfaces cell-based control systems with a continuously changing environment. Support for this concept has derived, in part, from investigation of the family of unique epidermal lipids [4,11–13]. These lipids are stored and secreted in vesicular structures [14] similar to the phospholipid-rich lamellar bodies of the pulmonary alveolar type II cell [15,16]. Like their counterparts in the lung, lipid secretory cells in the epidermis undergo progressive maturation, ultimately giving rise to differentiated cell types subserving barrier functions at the environmental interface [17,18].

In addition to the lipid "mortar," the stratum corneum also consists of polymeric "bricks;" i.e., corneocytes formed by terminal involution of lipid-laden keratinocytes in the upper stratum granulosum. A hallmark of this process, at the cellular level, is the spatially discrete and temporally abrupt formation of transitional cells

Table I. Average Corneocyte or Peridermal Cell Surface Area in the Late Fetal or Early Neonatal Rat<sup>a</sup>

Experiment	Age	Body Weight (g) (mean ± SE)	Area Examined	Number of Cells Examined	Average Cell Surface Area (µ²)
1	6 h	$6.9 \pm 0.1$	Dorsal epidermis	43	$1908 \pm 36$
2	12 h	$7.1\pm0.1$	Dorsal epidermis	59	$1846 \pm 35$
3	25 h	$7.5\pm0.1$	Dorsal epidermis	169	$1620 \pm 21$
4A	45 h	ND	Dorsal epidermis	41	$1587 \pm 28$
4B	45 h	ND	Ventral epidermis	75	$1802 \pm 33$
5	20-d gestation	$4.0 \pm 0.1$	Dorsal periderm	17	$4544 \pm 288$

<sup>a</sup> Tape-stripped samples were obtained from the dorsal or ventral epidermal surfaces as indicated. Corneocytes were examined on the third tape strip whereas periderm was examined on the first strip. Values for body weights and cell surface areas are means ± SEM. ND, not determined.

[5]. One measure of the rate of transitional cell formation, and, hence, the rate of cornification, is to count the increase in stratum corneum layer formation over a given period of time. For this purpose, we used the alkaline-expansion technique of stratum corneum analysis first described by Christophers and Kligman [19] and, later, applied by MacKenzie in his elegant demonstration of ordered epidermal structure [20,21].

Applying this method to the rat, prenatal and postnatal regression lines were generated (Fig 2), which allowed calculation of the rate of stratum corneum layer formation as a function of ontogenetic age. These data support the concept that birth marks a slowing of corneum production (from approximately one layer every 5 h during late gestation to one layer every 8 h after birth). This latter rate is consistent with a previous report by MacKenzie who measured an increase of six layers of corneum in neonatal rat backskin over the first 48 h after birth [9]. Similar differences are present when the results are calculated as a function of body weight (Fig 3). Figure 4 compares the overall rate of perinatal stratum corneum formation over the dorsal and ventral epidermis, respectively. Of interest, the ventral epidermis exhibited consistently fewer layers but the overall rate of increase was similar, suggesting a possible delay in the time of onset of ventral cornification *in utero*.

Application of the above method is clearly dependent upon assurance that sloughing of cells from the upper stratum corneum does not occur. Such losses would invalidate the counting procedure and result in indeterminate data. A major advantage of the perinatal rat model is that the periderm persists postnatally [9,22]. Earlier workers stated that the periderm detaches from the epidermal surface of the fetal pup 2-3 days before birth [23]. In this study, we have followed Stern *et al* [22] in ascribing a bilayer structure to the fetal periderm, the inner granular layer of which remains after birth. The postnatal persistence of the periderm, as clearly seen with the alkaline expansion technique (Fig 1), assures that the earliest layers formed *in utero* are still present at the time of postnatal counting; e.g., as late as day 3 after birth. The corneum layer subjacent to the periderm, therefore, contains the "oldest" corneocytes; i.e., those formed on day 19 to 20 of gestation.

At the cellular level, stratum corneum formation occurs, not one layer at a time, but one cell at a time. In this study, we used tape stripping and phase-contrast microscopy (Fig 5) to determine the average surface area of the corneocyte "bricks." According to this approach, superficial corneocyte dimensions can be used as a generic

 
 Table II.
 Effect of Exogenous EGF on Stratum Corneum Formation in the Neonatal Rat<sup>a</sup>

	Number of Stratum Corneum Layers	Body Weight (g)	Estimated Surface Area (cm²)
Control $(n = 11)$	$17.3 \pm 0.3 \\ 18.3 \pm 0.2^{b}$	$7.6 \pm 0.1$	30.2
EGF Rx $(n = 12)$		$7.0 \pm 0.2^{b}$	27.5

• Following birth, littermate pups received saline or a single dose of EGF (500 ng/g BW). Twenty-four hours later body weights were recorded and the number of layers of stratum corneum was determined. The surface area of the animals was calculated as described previously [10]. Values are means  $\pm$  SEM.

<sup>b</sup> p < 0.01, Student t test.

estimate of the size of new polymer units incorporated into the stratum corneum, and, consequently, kinetic models of cornification can be constructed (see *Materials and Methods*). This concept is based upon the assumption that the rate of formation of the stratum corneum, under conditions in which no desquamation occurs, is equivalent to the rate of production of transitional cells at the stratum corneum/granulosum interface.

The end result of terminal differentiation in the Malpighian layer of the epidermis is the production of transitional cells that, in the older organism, interdigitate to form the organized columns characteristic of slowly dividing, non-traumatized epidermis [24-26]. In the newborn rat, however, there is a lack of epidermal column formation, presumably due to increased cell turnover [9,22] and the rapid expansion of surface area associated with somatic growth [9,10]. As initially noted by MacKenzie [9] and confirmed by our data, the average size of the mature corneocyte does not increase following birth (Table I). An increase in corneocyte surface area is, of course, incompatible with the eventual formation of columns in which interdigitating cells must maintain equal transverse dimensions [27]. The fact that corneocytes subjacent to the periderm do not appear to expand with somatic growth raises important questions regarding the plasticity of the intercellular cementing substance and the regulation of intercorneocyte cohesion during development.

Focus on the transitional cell and the cornification process highlights some unusual aspects of terminal differentiation in the mammalian epidermis. In general, cell death is subdivided into two basic mechanisms: apoptosis and necrosis [28,29]. The process of cornification fits neither category neatly and appears, in some ways, similar to red cell senescence [30]. Certainly, in the epidermis, the mode of elimination of terminally differentiated cells is different from apoptosis or necrosis; i.e., sloughing to the environment versus local phagocytosis [29].

Nevertheless, there are a number of points in favor of considering mammalian cornification as a *forme fruste* of apoptosis: 1) epidermal cell death appears to be "programmed" and, like apoptosis, is an important mechanism in maintaining tissue homeostasis [31]; 2) there is evidence that endonuclease activation and DNA fragmentation occur in the terminally differentiating epidermis as they do in apoptotic cells [6]; 3) the high levels and distribution of epidermal calcium [10,32] support the concept that this ion is involved as a potential trigger mechanism in cellular involution [29,33]; 4) there is an absolute lack of inflammatory response similar to apoptosis [28,29]; 5) cornification is characterized by shrinkage or condensation necrosis rather than cell swelling [34]; 6) there is recent evidence that, like apoptosis, cornification is preceded by specific gene activation in terminally differentiating keratinocytes [35]; 7) the time frame for transitional cell formation appears to be very rapid (minutes to hours) consistent with the duration of the apoptotic cell [29]; 8) membrane blebbing is a classic finding in the terminally differentiating apoptotic cell and there is marked plasticity of the transitional cell boundary accompanying corneocyte formation (see Fig 4 in [36]); and, finally, 9) the rate of transitional cell formation, like the apoptotic cell, appears to be under hormonal control ([29], Table II).

In this regard, exogenous EGF has been demonstrated to increase





В



**Figure 6.** Scanning electron micrographs of the dorsal epidermis of 7day-old rats following neonatal EGF Rx. Littermate rat pups received saline or the indicated doses of EGF on postnatal days 0-3. Animals were killed at 1 week of age and the dorsal epidermal surface examined by low-power scanning electron microscopy. EGF induces a dose-dependent hyperkeratosis of the interfollicular epidermis and a characteristic curvilinear growth pattern of monotrichs. *Bar*, 230  $\mu$ .

apoptosis associated with the catagen phase of the hair-growth cycle [37]. Our data are consistent with an effect of EGF to increase the rate of transitional cell formation. The same growth factor, therefore, can augment both the rate of epidermal proliferation, as assessed by standard tests of thymidine incorporation [38,39], as well as the rate of terminal differentiation, as assessed by tests of stratum corneum formation (Table II). Whether EGF is increasing epidermal cell migration in addition to its mitotic action [40] is undetermined but consistent with the data. Repetitive treatment of the neonatal rat with EGF on postnatal days 0-3 results in hyperkeratosis of the interfollicular epidermis at 1 week of age (Fig 6A - C). An unusual feature of this response is the development of curvilinear monotrichs [41,42]. This epidermal syndrome is evoked by pulsed administration of a purified growth factor during a critical developmental window [7,39,43]. In our view, this model seems amenable to investigating a variety of questions in epidermal biology ranging from the "mitotic pressure" hypothesis [40] to the control of corneocyte cohesion and the regulation of desquamation [11].

In summary, morphometric approaches have been utilized in the perinatal rat to investigate factors influencing the rate of stratum corneum formation. This rate is maximal prior to birth and diminishes shortly thereafter. A quantitative estimate of transitional cell kinetics has been offered for the newborn period. According to the model proposed, normal corneum synthesis near birth requires keratinocytes to cornify at an approximate rate of 3 cells/second/cm<sup>2</sup> of body surface. Treatment with exogenous EGF augments that rate of terminal differentiation of perinatal rat epidermis. These findings support the use of the perinatal rat as a model system for investigating factors involved in the complex process of stratum corneum biogenesis.

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